JCB Comment

Traffic control: p120-catenin acts as a gatekeeper to control the fate of classical cadherins in mammalian cells

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Proteins of the p120 family have been implicated in the regulation of cadherin-based cell adhesion, but their relative importance in this process and their mechanism of action have remained less clear. Three papers in this issue suggest that p120 plays a key role in maintaining normal levels of cadherin in mammalian cells, and that it may do so by regulating cadherin trafficking (Chen et al., 2003; Davis et al., 2003; Xiao et al., 2003).

To assemble into organized tissues, cells must interact with one another and with the extracellular matrix. Cell–cell adhesion is initiated and maintained by adherens junctions (for review see Angst et al., 2001). Within these junctions, transmembrane cadherins bind homophilically to join neighboring cells (Fig. 1 A). The distal portion of the cadherin cytoplasmic tail binds β-catenin, which in turn recruits α-catenin, and thereby links the adhesive complex to the actin cytoskeleton. These three proteins are core components of adherens junctions: each is essential for cell adhesion and for tissue architecture. This view of the junction is static, but in reality cell adhesion is carefully and continuously adjusted. Changes in adhesion and in the connection of cadherins to the actin cytoskeleton allow cells to carry out the complex events of embryonic development, tissue remodeling, and wound repair. In fact, even cultured epithelial cells turn over junctional proteins with a half-life of about 5 h (Shore and Nelson, 1991), and this must be balanced with the assembly of new proteins into the junction. The mechanisms by which adhesion and cytoskeletal connections are regulated remain largely mysterious.

One candidate regulator of adhesion is p120, the founding member of the p120 protein family (for review see Anastasiadis and Reynolds, 2000). p120 was initially identified as a substrate of the oncogenic nonreceptor tyrosine kinase Src. Src activation triggers extensive changes in cell–cell and cell–matrix adhesion, and p120 was considered a possible mediator of some of these effects. p120 is also a target of receptor tyrosine kinases. p120 is a distant relative of β-catenin, sharing with it a set of protein–protein interaction motifs known as Arm repeats. Like β-catenin, p120 binds to the cytoplasmic tail of all classic cadherins, but p120 binds to the juxtamembrane (JM) rather than the distal region where β-catenin binds (Fig. 1 A).

Indirect evidence for a role of p120 in adhesion regulation came from a series of studies examining the role of the JM region of the cadherin tail. All of these studies suggested that the JM region plays an important role, but the nature of this role differed between the different studies. For example, Ozawa and Kemler (1998) found that deletion of the JM domain restored adhesion to a partially disabled cadherin, implying that it negatively regulated adhesion. They suggested it might do so by regulating cadherin dimerization. In contrast, Yap et al. (1998) found that the JM region was essential for strong adhesion of cells to cadherin-coated substrates, suggesting that it plays a positive role in adhesion. They hypothesized that the JM domain might regulate cadherin clustering. These contradictory effects on adhesion were surprising, but might be rationalized if, for example, binding of p120 to the JM region had different effects on adhesion that depend on distinct p120 phosphorylation states in different cell types. However, since these and similar studies manipulated the cadherin JM domain, they did not directly implicate p120. Other molecules (e.g., the presenilin transmembrane proteases and the ubiquitin ligase Hakai) also bind to the JM region, so a more direct test of p120's role was needed.

The first direct test of p120 function in adhesion was published here last year by the Reynolds laboratory (Ireton et al., 2002). Genetic analysis of the mammalian p120 family is complicated by the presence of four closely related family members: p120, ARVCF, δ-catenin, and p0071 (for review see Anastasiadis and Reynolds, 2000). However, these differ in their tissue distribution, encouraging the Reynolds group to look for a cell line lacking p120 expression. After an

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Abbreviations used in this paper: JM, juxtamembrane; siRNA, small interfering RNA.

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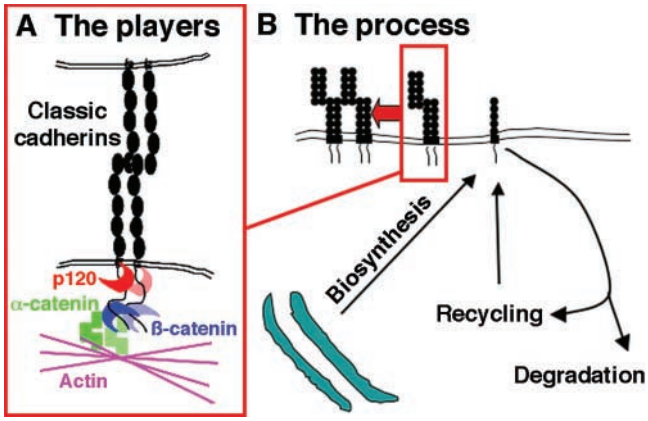

Figure 1. **The cellular itinerary and fate of classical cadherins.** (A) Adherens junctions are assembled around classic cadherins. Their extracellular domains mediate homophilic cell–cell adhesion. The distal regions of their cytoplasmic tails bind β-catenin, which in turn binds α-catenin. α -catenin links the junction to the actin cytoskeleton via direct and indirect interactions. The juxtamembrane domain of the cadherin cytoplasmic tail binds proteins of the p120 family. (B) The surface expression of E-cadherin is one facet of a complex trafficking network. Upon delivery to the cell surface, classical cadherins may adopt either of two fates. Productive homophilic ligation and attachment to the cytoskeleton allows cadherins to be retained at the cell surface, supporting lateral clustering and assembly of cadherins into adherens junctions (red arrow). Alternatively, cadherins may be endocytosed. They then can either be trafficked back to the cell surface (via a post-Golgi recycling pathway) or ultimately targeted for degradation, at least in part via lysosomes. Cadherins delivered to the cell surface can thus derive either from newly synthesized material or via the recycling pathway. p120 appears to play a key gatekeeper function in determining the fate of cadherins after delivery to the surface. When incorporated into the cadherin complex p120 can also potentially coordinate a range of other activities, including surface adhesion, cadherin clustering, and cell signaling.

extensive search, they found a tumor cell line that had little or no detectable wild-type p120 (Ireton et al., 2002). Unlike most other epithelial cell lines, these "*p120* mutant" cells no longer formed compact adherent colonies, although cell interactions were not completely abolished. This cell line also had much lower levels of E-cadherin than most epithelial cell lines. Both cell adhesion and cadherin levels were reverted back to normal by transfection of wild-type *p120*. These data strongly suggest that p120 promotes cell adhesion by potentiating cadherin levels or activity in some way. However, the adhesive phenotype of the *p120* mutant cell line also could be rescued by overexpression of either wild-type E-cadherin or of an E-cadherin that has a mutation in the p120-binding site, suggesting that E-cadherin retains residual function in the absence of p120, if it can be expressed at reasonable levels.

Next, two studies were published that examined the role of p120 and the JM region of E-cadherin in *Drosophila*. Genetic analysis of *p120* in flies is simplified by the fact that there is only a single p120 family member. Myster et al. (2003) generated null mutations in it, and examined the mutant phenotype. To their surprise, animals completely lacking the single p120 family member were viable and fertile, and showed no diminution in the levels of E-cadherin or other junctional proteins. However, fly p120 does positively promote cell adhesion, as mutations in *p120* strongly enhance the effects of reducing the dose of E-cadherin. In parallel work, Pacquelet et al. (2003) showed that mutating the p120 binding site on the JM domain of *Drosophila* E-cadherin had no discernable effect on its function, as the mutant version rescued animals lacking endogenous E-cadherin. These data suggest that, in *Drosophila* at least, p120 is not a core component of the adherens junction, but rather acts as a positive regulator of adhesion. A test of *p120* function in the nematode *C. elegans* using RNA interference came to a similar conclusion—knockdown of the single p120 family member had no effect in a wild-type animal, but p120 knockdown enhanced the phenotype of a weak mutation in *α-catenin* (Pettitt et al., 2003). These data are consistent with the work of Ireton et al. (2002), but suggest that in *Drosophila* and *C. elegans* the role of p120 may not be as critical as it is in mammalian cells, perhaps because other proteins work by parallel mechanisms to regulate adhesion.

These studies set the stage for the three papers in this issue. They ask two important questions: (1) does p120 play a critical and general role in cell adhesion and cadherin regulation in mammalian cells, and (2) by what mechanism(s) does it regulate cadherin function? Although the data of Ireton et al. (2002) suggested that p120 might regulate E-cadherin levels, these findings were confined to a single cell line, and that was a tumor cell line that also has a *β-catenin* mutation. Both Davis et al. (2003) and Xiao et al. (2003) extend and test the generality of this observation by using small interfering RNAs (siRNAs) to knockdown p120 expression in other cell lines. Davis et al. (2003) examined several cell lines with similar results: reduction in p120 levels led to a strong reduction in steady-state cadherin levels, associated with loss of cell–cell adhesion. Further, they found that these effects were not specific for E-cadherin—p120 knockdown also destabilized VE-cadherin and N-cadherin. Similar effects on VE-cadherin were seen by Xiao et al. (2003). Interestingly, expression of the classic cadherin-binding p120 family members δ-catenin and ARVCF (but not the more distant desmosomal relative plakophilin 3) could also rescue cadherin stability, implying that the p120 family members may all share this function. Both groups also found that as they added back more and more p120, the levels of cadherin rose in parallel, suggesting that p120 acts as a rheostat to regulate cadherin levels. Together, these data persuasively argue that in mammalian cells p120 is critical to maintain steady-state cadherin levels and thus support cell adhesion, an influence that extends across classical cadherins and cell types.

How then might p120 regulate cadherin accumulation at the cell surface? The reports in this issue suggest that p120 is an important regulator of cadherin trafficking. As is the case with other transmembrane proteins, the synthesis, recycling, and degradation of cadherins are linked by a complex meshwork of intracellular trafficking pathways (Fig. 1 B). Newly synthesized cadherins are transported from the Golgi apparatus to the plasma membrane via a pathway that involves targeting information in the cadherin cytoplasmic tail (Miranda et al., 2001) and requires association with β-catenin (Chen et al., 1999). Surface cadherins are eventually en-

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docytosed, but after internalization they can either be rapidly recycled back to the cell surface via a post-Golgi transport pathway (Le et al., 1999) or targeted for lysosomal degradation (Xiao et al., 2003). The precise relationship between the recycling and degradative pathways is not well-understood but the two are likely to be linked.

The earlier work of Ireton et al. (2002) had suggested that loss of p120 reduces the half-life of E-cadherin. Davis et al. (2003) extend this work, using metabolic labeling studies to show that the synthesis rates of E-cadherin are not appreciably affected by loss of p120, but cadherin degradation is accelerated. Further, p120 knock down did not seem to affect the delivery of newly synthesized E-cadherin to the cell surface (Davis et al., 2003). Instead, after delivery E-cadherin was rapidly lost from the cell surface, suggesting that p120 is necessary for its stable retention at the plasma membrane. The Kowalczyk group found that VE-cadherin is internalized in the absence of p120 and is trafficked to lysosomes (Xiao et al., 2003). Furthermore, down-regulation of E-cadherin could be reversed when cells were treated with inhibitors of lysosomal activity. These findings are thus consistent with an emerging model where, without p120, classical cadherins cannot be stably retained at the cell surface and thus are rapidly internalized to be eventually trafficked to lysosomes for degradation.

There are at least three nonexclusive mechanisms by which p120 might determine the balance between synthesis, retention, and degradation of cadherins. Davis et al. (2003) and Xiao et al. (2003) suggest that p120 could interact with the endocytic machinery to inhibit cadherin endocytosis. Cadherins can be internalized via both clathrin-mediated (Palacios et al., 2001) and clathrin-independent (Paterson et al., 2003) pathways. Without knowing which pathway is used, the precise role of p120 in this process is difficult to evaluate at present. Further, we do not yet know the signals that target classical cadherins for endocytosis and degradation. It is interesting that proteosomal inhibitors could reverse E-cadherin down-regulation after p120 knockdown (Davis et al., 2003), suggesting a role for protein ubiquitination (Fujita et al., 2002). However, numerous studies demonstrate that cadherin mutants that fail to bind p120 can be stably expressed, suggesting that inhibiting endocytic uptake alone is unlikely to fully explain the effects on adhesion of p120.

The effect of p120 on cadherin levels might reflect a more direct role in cell adhesion. Although studies of the role of the JM domain in adhesion came to varied conclusions, many cadherin mutants that cannot bind p120 are poorly adhesive (Thoreson et al., 2000), perhaps through changes in lateral clustering of cadherins, which strengthens adhesion, or by altered cadherin-activated signaling (Goodwin et al., 2003). Notably, cadherin internalization is increased when cell–cell contacts are broken (Le et al., 1999), suggesting that productive adhesion may prevent cadherin endocytosis. If productive adhesion is lost when p120 is depleted, then this would be predicted to increase the pool of cadherins available for endocytosis, leading to increased net internalization of cadherin.

The third paper in this issue suggests an additional possibility: that p120 participates in the transport of cadherins to the cell surface (Chen et al., 2003). Cadherin-containing vesicles have been shown to move on microtubule tracks toward cell–cell contacts (Mary et al., 2002). Chen et al. (2003) now demonstrate that p120 can bind conventional kinesin, and in movies of GFP-tagged p120, they found that p120 and kinesin decorate cadherin-containing vesicles moving along microtubules toward cell–cell contacts. Moreover, by using either cadherin mutants incapable of binding p120 or p120 mutants that cannot bind kinesin, they showed that disruption of the cadherin/p120/kinesin linkage retarded the rate at which cadherins reaccumulated in cell–cell contacts that had been broken by depleting extracellular calcium (the well-known "calcium switch" assay). Although they do not exclude a role for p120 in regulating cadherin retention at reforming contacts, these findings suggest the intriguing possibility that p120 participates in the kinesin-driven transport of cadherin to the cell surface. Loss of p120 would reduce the efficiency with which cadherins are transported to the cell surface in the outward-bound limb of a recycling pathway, and perhaps also in the biosynthetic pathway. Decreased outward transport might then promote cadherin degradation by shunting internalized cadherins toward a lysosomal fate.

These three papers go a long way to establishing a key role for p120 in regulating cadherin expression in mammalian cells. They strongly suggest that p120 serves as a gatekeeper, determining whether cadherins are stably retained at the cell surface, or are endocytosed for eventual degradation. Moreover, the work of Chen et al. (2003) draws our attention to the capacity for p120 to influence the kinetics, and perhaps the itinerary, of cadherin transport as it reenters cells. Clearly, much remains to be learned. In particular, regulation of cadherin levels is unlikely to be the sole mechanism by which p120 determines adhesion, since p120-uncoupled cadherin mutants can be effectively expressed at the cell surface but are poorly adhesive. Likewise, these data and the ability of flies and worms to live without p120 suggest that other mechanisms for regulating cadherin turnover are likely to exist. Finally, p120 can also intersect with a range of signal transduction molecules including Rho-family GTPases and the transcription factor Kaiso, none of which we have discussed here. We thus need to understand how p120 and other potentially redundant machinery might influence the relationship between surface adhesion, signaling, and endocytosis. Mapping p120's interactions and identifying the rules that govern them will be an interesting challenge in the next few years.

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